Advancing Male Contraception:

Reviewing Existing Methods and the Innovative Approach of YCT-529 through Retinoic Acid Receptor-α Inhibition

Capstone Project

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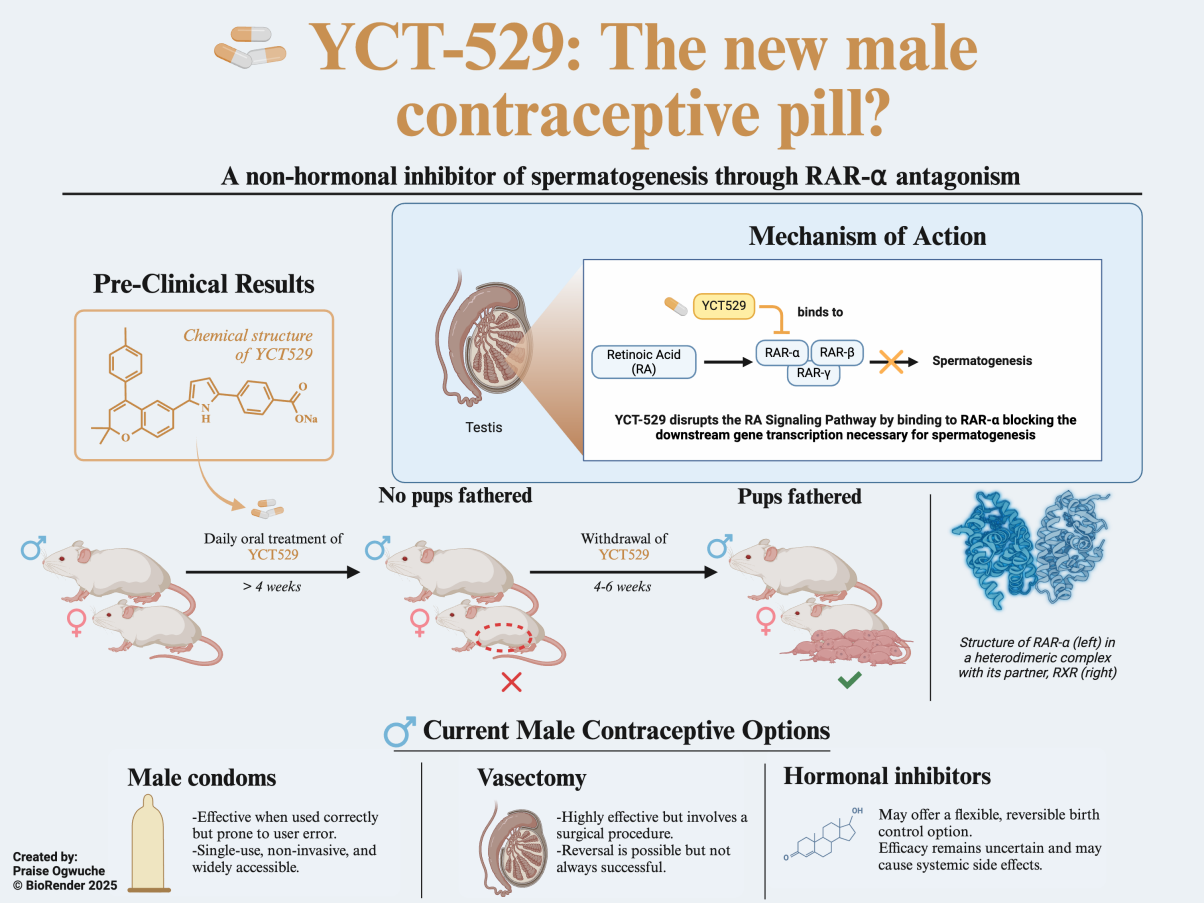
Contents

Abstract

YCT-529, chemically known as 4-[5-[2,2-dimethyl-4-(4-methylphenyl)chromen-6-yl]-1H-pyrrol-2-yl]benzoic acid, is a highly selective non-hormonal oral male contraceptive targeting retinoic acid receptor alpha (RARα) to disrupt spermatogenesis. This review examines YCT-529's structure, comprising a chromene core, pyrrole linker, and benzoic acid moiety, which confers high specificity for RARα while reducing off-target interactions with RARβ and RARγ. The molecule's rigid structure and hydrogen bonding enhance stable binding to the receptor's ligand-binding domain, blocking coactivator recruitment essential for gene transcription. Preclinical murine studies demonstrated a 99% reduction in sperm production with full reversibility within 4 weeks and no effect on testosterone levels or sexual function [50]. In vivo pharmacokinetic studies in mice showed a half-life of 11 hours, with peak plasma levels of 2.1 µM and significant testicular exposure [50]. Oral doses of 10–20 mg/kg/day in non-human primates achieved 100% suppression of sperm production without systemic toxicity [40]. Following these promising results, YCT-529 advanced to Phase 1 clinical trials in London, assessing safety, tolerability, and pharmacokinetics in healthy male volunteers. In-silico modeling using PBPK simulations predicted variations in systemic retention across different populations, refining dosing strategies. If successful, YCT-529 could revolutionize male contraception, offering a safe, reversible alternative that addresses longstanding contraceptive inequities.

Keywords: YCT-529, non-hormonal male contraceptive, retinoic acid receptor alpha (RARα), spermatogenesis, pharmacokinetics, PBPK modeling, contraceptive pharmacotherapy



Graphical Abstract: Summary of YCT-529's mechanism, preclinical results, and comparison to existing male contraceptives. Created using BioRender. Available here.

Introduction

The lack of effective male contraceptive options has perpetuated an imbalance in reproductive responsibility, with the burden disproportionately placed on women. Despite significant advancements in contraception, unintended pregnancies remain a global issue, comprising nearly half (approximately 213 million) of all pregnancies annually, particularly affecting underserved populations [38]. Existing male contraceptive methods, such as condoms and vasectomy, offer limited options: condoms are highly user-dependent and have a failure rate of 15% with typical use, while vasectomy is a largely irreversible surgical intervention [40]. The absence of a widely accessible, reversible male contraceptive continues to reinforce gendered inequities in reproductive health.

A promising solution lies in the inhibition of retinoic acid receptor alpha (RARα), a key nuclear receptor that regulates spermatogenesis. Genetic studies have demonstrated that RARα knockout mice are viable but sterile due to the failure of sperm maturation, highlighting its necessity in male fertility [40]. This finding has driven the development of RARα-specific antagonists, such as YCT-529, a novel small-molecule inhibitor designed to selectively disrupt spermatogenesis without altering systemic hormone levels [40]. Unlike hormonal male contraceptives, which rely on suppressing the hypothalamic-pituitary-gonadal (HPG) axis and often cause side effects like weight gain, mood disturbances, and reduced libido, non-hormonal agents such as YCT-529 provide a targeted approach with minimal systemic effects [40]. Preclinical studies have demonstrated that YCT-529 effectively induces reversible infertility in murine models, achieving a 99% reduction in sperm count while preserving testosterone levels and sexual function [50].

While the pharmacological advancements in YCT-529 signify a major breakthrough, the broader implications of male contraception must be addressed. Historically, female contraceptive methods have undergone extensive research and development, while male-focused solutions have remained stagnant due to biological, regulatory, and sociocultural barriers. The disparity in contraceptive responsibility has led to significant physical, emotional, and financial burdens for women, as well as moral and legal dilemmas regarding unintended pregnancies [38]. Male contraceptive options, if widely accepted, have the potential to reshape gender dynamics in family planning, fostering shared responsibility and alleviating existing inequities.

This review aims to provide a comprehensive analysis of the biochemical, pharmacological, and clinical landscape of YCT-529, highlighting its mechanism of action as a selective RARα antagonist, its efficacy in preclinical models, and its potential implications in human contraception. The review is structured into three sections: (1) the current landscape of male contraceptive methods and their limitations, (2) the role of RARα in spermatogenesis and the rationale behind its inhibition, and (3) an in-depth examination of YCT-529, including its chemical properties, pharmacokinetics, and preclinical findings.

By integrating molecular biology, medicinal chemistry, and contraceptive policy considerations, this review underscores the importance of developing safe, effective, and reversible male contraceptives. The emergence of YCT-529 represents a critical step toward achieving contraceptive equity and expanding reproductive choices for men.

Note: For the sake of clarity, this review uses the terms "male," "men," "female," and "women" interchangeably; however, these terms alone do not sufficiently convey the full spectrum of gender diversity, and their use here is not intended to exclude individuals with diverse gender identities.

Background Information - Contraception

Contraception encompasses a range of methods designed to prevent pregnancy, allowing individuals to manage fertility and plan families while maintaining personal autonomy [4]. These methods include barrier devices, hormonal interventions, and surgical procedures, each varying in effectiveness, mechanism, and ethical considerations, particularly concerning informed consent and access. Hormonal contraceptives like Levonorgestrel and Ulipristal Acetate prevent ovulation or inhibit sperm production, whereas non-hormonal methods, such as copper IUDs and condoms, create physical barriers without altering systemic hormone levels [23]. The diversity of contraceptive options provides flexibility in addressing individual safety concerns and minimizing systemic side effects.

The development of non-hormonal male contraceptives, such as YCT-529, represents a significant advancement in reproductive health. These innovations aim to minimize adverse effects, addressing historical imbalances where women have disproportionately borne the health risks associated with hormonal contraceptives [38]. By fostering shared responsibility in family planning, non-hormonal options promote gender equity and support psychological well-being. Expanding access through strategic incentives and choice architecture normalizes male participation, creating a feedback loop that accelerates societal acceptance and innovation in contraceptive technology [37].

Aim of Review

This review explores receptor-targeted approaches in spermatogenesis as a foundation for non-hormonal male contraceptives. By assessing the historical and modern landscape of male contraception, it contextualizes the significance of receptor-based methods. Designed for both scientific and public engagement, this review highlights the potential of emerging technologies to transform male contraception and improve family planning.

Current State Of Understanding

The historical development of male contraceptives reveals a gradual evolution of options and approaches, reflecting broader societal, technological, and medical changes over the decades.

Condoms

Condoms have long been a cornerstone of contraception and STI prevention, evolving from early linen and animal intestine sheaths to modern latex and polyurethane versions [33]. Latex condoms provide reliable protection against STIs such as HIV, gonorrhea, and chlamydia, achieving 98% efficacy in preventing pregnancy with perfect use, though this drops to 85% with typical use due to application errors. Despite their accessibility and non-invasive nature, issues such as breakage, incorrect use, latex allergies, and reduced sensation limit their effectiveness. Alternative materials like polyurethane address allergy concerns but come at a higher cost. Future advancements, such as graphene-infused and microbicidal condoms, aim to enhance durability, protection, and user experience [33].

Vasectomies

Vasectomy is a permanent sterilization procedure that involves severing and sealing the vas deferens to prevent sperm from entering the ejaculate. It is highly effective, with a 99.7% success rate and a low complication rate of 1%–2% [27, 52]. The procedure, performed using conventional or no-scalpel techniques, preserves testosterone production, libido, and erectile function. While vasectomy is less invasive than female sterilization and does not require daily adherence, its permanence raises psychological considerations, as reversals are costly and not always successful. Though fears about prostate cancer have been largely disproven, risks include infection, sperm granulomas, and rare cases of chronic pain, necessitating thorough pre-procedure counseling [52].

Hormonal Male Contraceptives

Hormonal male contraceptives suppress spermatogenesis by modulating the hypothalamic-pituitary-gonadal (HPG) axis. Since the 1970s, they have utilized exogenous testosterone, often with progestins, to inhibit gonadotropins such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both essential for sperm production [61]. Studies by the NICHD and WHO confirmed that hormonal contraceptives could achieve efficacy comparable to female methods with minimal side effects. The NES/T gel, combining segesterone acetate and testosterone, effectively suppresses gonadotropin secretion while preserving secondary sexual characteristics and libido. Clinical trials by Ilani et al. [22]

demonstrated that daily NES/T application significantly reduced sperm concentration, achieving 89% efficacy. Phase IIb trials involving 400 couples globally continue evaluating its real-world contraceptive potential [1].

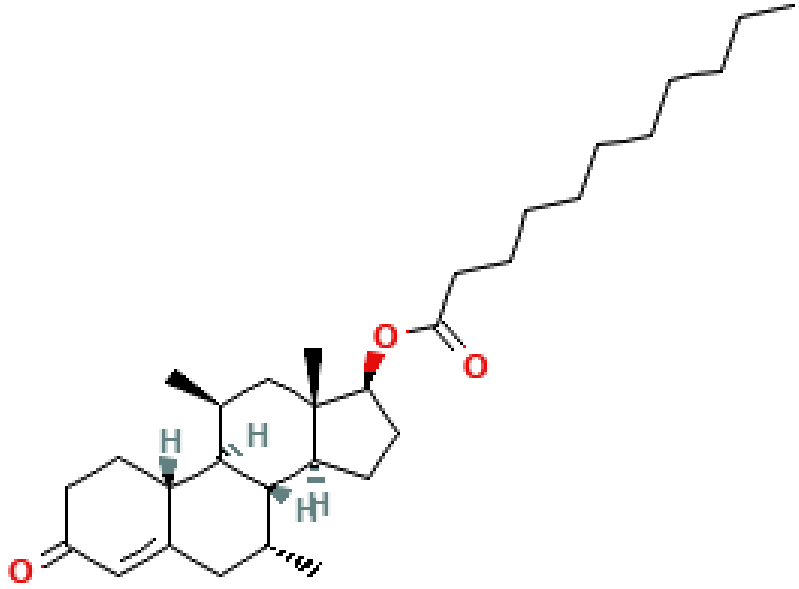


Figure 1: *Structure of dimethandrolone undecanoate (DMAU), a synthetic testosterone derivative. The undecanoate ester at C17 enhances lipid solubility and prolongs metabolic stability. By binding androgen and progesterone receptors, DMAU suppresses gonadotropins, reducing LH and FSH secretion while avoiding estrogenic side effects. Adapted from [35].*

Dimethandrolone undecanoate (DMAU) and 11β-methyl-19-nortestosterone dodecylcarbonate (11β-MNTDC) are promising oral hormonal contraceptives. Figure 1 depicts DMAU's structure, highlighting its undecanoate ester, which enhances lipid solubility and metabolic stability. DMAU selectively binds to androgen (AR) and progesterone (PR) receptors, suppressing gonadotropins and reducing LH and FSH secretion [31]. Studies indicate that oral DMAU, taken with high-fat meals, achieves effective suppression at doses of 200 mg or higher [3]. Phase I trials by Thirumalai et al. [58] confirmed significant sperm suppression at 400 mg with minimal side effects, positioning DMAU as a leading candidate for daily male contraception.

The NES/T gel and oral hormonal agents offer non-invasive, reversible contraceptive options with minimal long-term health risks compared to permanent methods like vasectomy. However, adherence, hormonal absorption consistency, and user acceptance remain challenges [31]. Side effects such as skin irritation with NES/T and libido fluctuations with oral agents require further refinement. Clinical research continues optimizing formulations to enhance efficacy, user adherence, and tolerability.

As these technologies advance through phase II and III trials, they provide a viable path to expanding male contraceptive options. Addressing adherence, safety, and cultural

acceptance remains critical to their widespread adoption and success [1, 61].

RAR Antagonists and YCT-529

Therefore, the quest for non-hormonal male contraceptives arises from the limitations of existing methods. Hormonal approaches can cause mood changes, libido fluctuations, and require strict adherence [43, 38]. This review explores retinoic acid, a vitamin A metabolite essential for sperm development, as a target for non-hormonal contraception [40].

RAR Antagonist Experiments and other RARα Antagonists

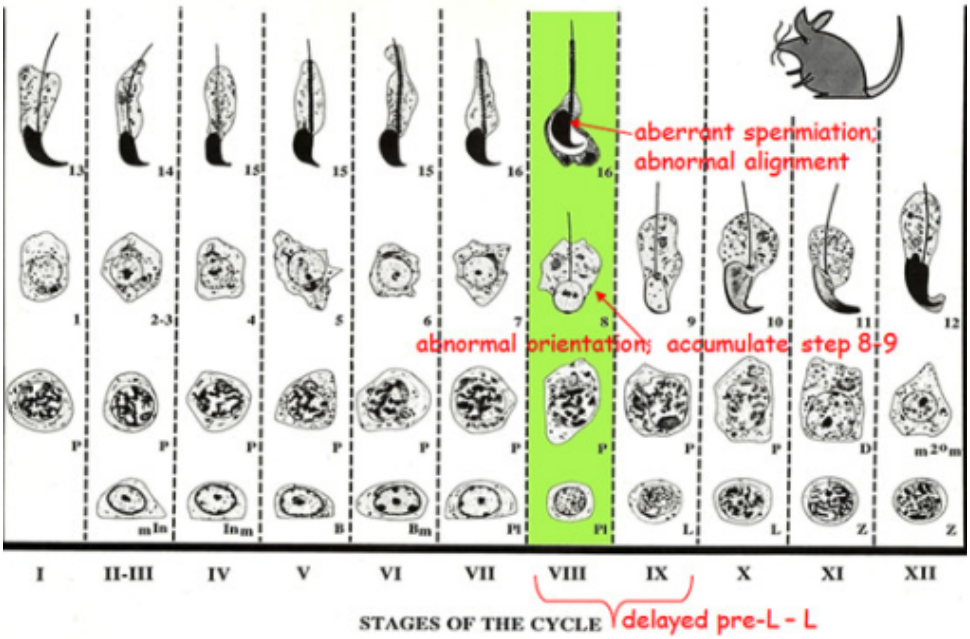
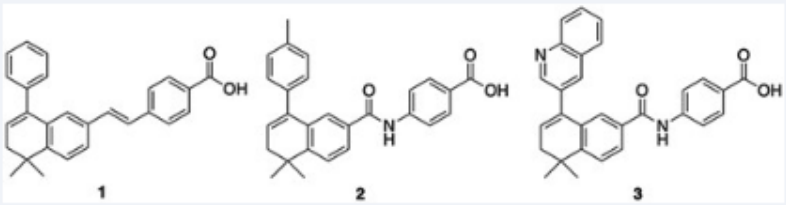


Figure 2: *Spermatogenic cycle disruptions in RARα-deficient testes, with stage VIII–IX showing abnormal spermiation, misaligned spermatids, and arrested cell progression. Red arrows highlight affected cell types. Adapted from Russell et al. [46].*



	Antagonism IC_{50} (nM)[a]			K_d (nM)		
Compound	**RARA**	**RARB**	**RARG**	**RARA**	**RARB**	**RARG**
1 (BMS-189453)	200	200	200	—	—	—
2 (BMS-189532)	—	—	—	27	1020	3121
3 (BMS-195614)	500	5000	10 000	—	—	—

[a]Transactivation assay.

Figure 3: *Structures and RAR antagonism profiles of BMS-189453, BMS-189532, and BMS-195614, showing IC_{50} values across RAR isoforms. Adapted from Noman et al. [40].*

Noman et al. [40] investigated BMS-189453 as a pan-RAR antagonist for male contraception, leveraging its known testis-specific effects. Oral administration (5 mg/kg for 7 days) in mice disrupted spermatogenesis, inducing spermatid misalignment, impaired sperm release, and germ cell sloughing, closely resembling Vitamin A-deficient (VAD) and RAR$\alpha^{-/-}$ phenotypes (Figure 2). Mating studies confirmed sterility was reversible, with no adverse effects on testosterone or libido [11]. Extending treatment (5 mg/kg for 2 weeks, 2.5 mg/kg for 4 weeks) prolonged sterility while preserving fertility recovery (Figure 3). Toxicological screening showed no systemic toxicity, and offspring from recovered males exhibited normal reproductive health [11]. These findings reinforced the feasibility of reversible male contraception via retinoid pathway disruption.

Further trials by Noman et al. [40] optimized BMS-189453 dosing for sustained sterility while ensuring fertility restoration. Administering 1 mg/kg for 4–16 weeks resulted in complete but reversible sterility, with faster recovery in lower-dose, longer-duration regimens. Testosterone remained stable, preserving libido and endocrine function. Gene expression profiling suggested ATP-binding cassette efflux and solute carrier influx transporters contributed to recovery kinetics [13]. These results underscored the balance between contraceptive efficacy and reversibility in RAR antagonism.

To minimize off-target effects, Noman et al. [40] explored RARα-selective antagonists BMS-189532 and BMS-195614 [55, 12]. Despite potent in vitro RARα inhibition (IC_{50} = 27 nM for BMS-189532), oral administration (2 mg/kg and 10 mg/kg for 7 days) failed to induce sterility, with no significant testicular abnormalities observed (Figure 3). Poor bioavailability, rapid hepatic metabolism, and high plasma protein binding impaired sys-

temic exposure. Intravenous administration resulted in stronger spermatogenic effects, confirming that oral absorption was the limiting factor. These findings highlighted the need for structural modifications to enhance bioavailability, a challenge ultimately addressed by YCT-529's design.

By demonstrating both the efficacy and limitations of pan-RAR and RARα-selective antagonists, these studies shaped the development of next-generation inhibitors with optimized pharmacokinetics, paving the way for YCT-529 as a clinically viable non-hormonal male contraceptive.

The Medicinal Chemistry of RAR Inhibitors

The medicinal chemistry of RAR inhibitors has evolved significantly, refining how molecular modifications influence receptor activity and selectivity. Early studies on all-trans-retinoic acid (ATRA) revealed that restricting its flexible polyene chain into a rigid aromatic ring enhanced receptor affinity. This led to TTNPB, a synthetic RAR agonist with improved stability (Figure 4). Further modifications introduced a C8 hydrophobic residue, shifting the receptor from an active to inactive state, enabling the development of antagonists like BMS-189453. These modifications demonstrate how targeted changes at the molecular level dictate receptor function.

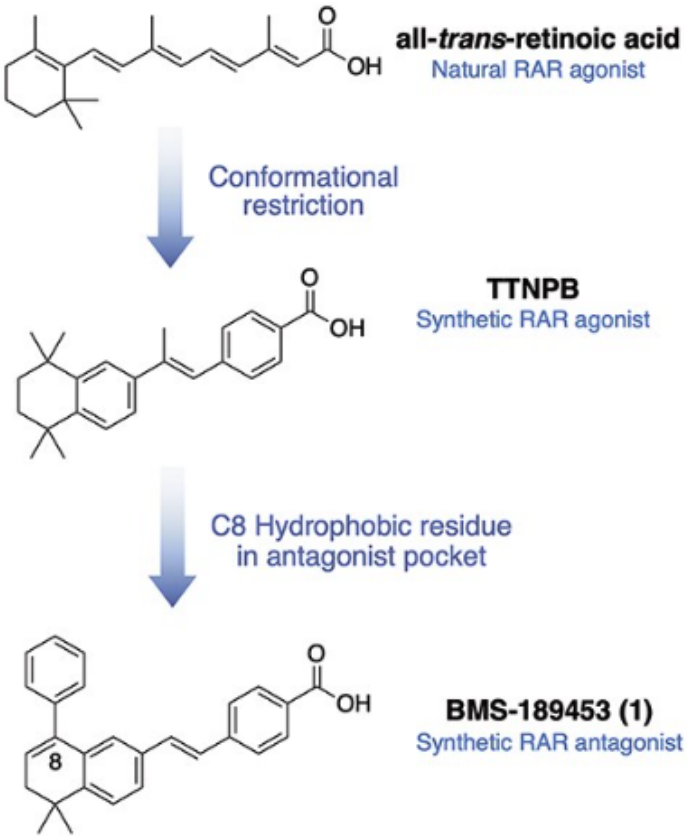


Figure 4: *Progression from ATRA to synthetic RAR ligands, illustrating structural changes that transition agonists to antagonists. Adapted from Noman et al. [40].*

RAR agonists such as Am580 stabilize an active receptor conformation that recruits coactivators, promoting gene transcription. In contrast, antagonists like BMS-189614 and BMS-204493 shift the ligand-binding domain (LBD) into an inactive conformation, blocking coactivator recruitment (Figures 5A–C). BMS-189614 displaces the helical region, while BMS-204493 reinforces this disruption, ensuring the receptor remains inactive. These interactions illustrate how structural variations influence receptor activation.

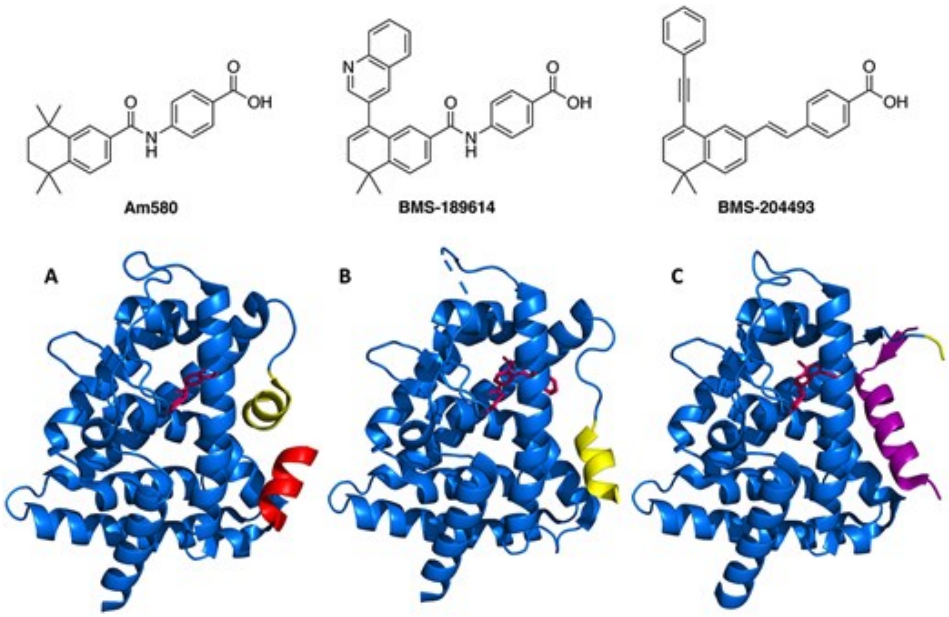


Figure 5: *Structures of RAR agonists and antagonists showing their effects on receptor conformation. Adapted from Noman et al. [40].*

RARα selectivity is largely determined by Ser232, a polar residue within its LBD that is absent in RARβ and RARγ. This unique binding environment allows selective ligands to form hydrogen bonds with Ser232, enhancing RARα affinity while limiting off-target interactions (Figure 6). In contrast, RARβ and RARγ contain alanine at this position, creating a less polar environment that reduces affinity for RARα-selective compounds. Ro 41-5253 exploits this distinction, showing significantly lower IC_{50} values for RARα compared to other isoforms (Figure 8). These findings highlight how single-residue differences drive receptor selectivity.

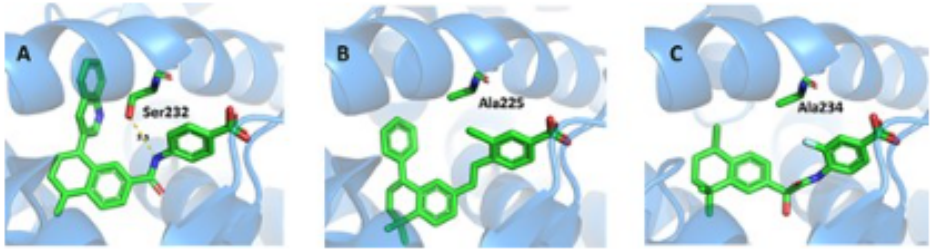


Figure 6: *Structural differences in RAR isoforms, showing how Ser232 in RARα enables selective ligand binding. Adapted from Noman et al. [40].*

Chromene and tetrahydronaphthalene derivatives further demonstrate how fine-tuned structural modifications enhance receptor specificity. AGN 193109 and its analogs introduce steric and electronic adjustments that strengthen RARα selectivity (Figure 7). Among them, compound 7 exhibits the highest affinity for RARα (4 nM), showing how

ligand optimization refines receptor interactions.

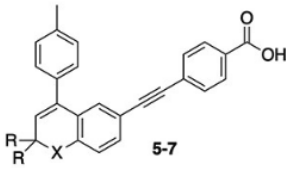
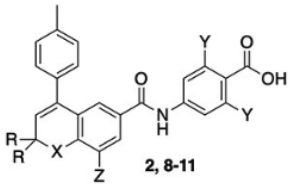
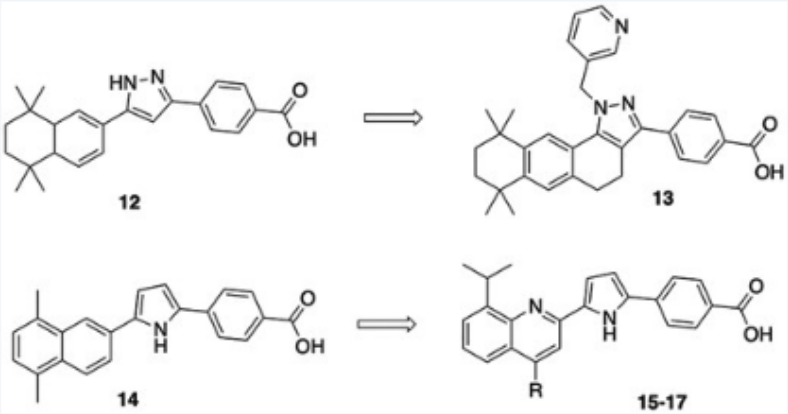
					K_i (nM)		
Compound	**X**	**R**	**Y**	**Z**	**RARA**	**RARB**	**RARG**
 **5-7**							
ATRA					15	13	18
5 (AGN 193109)	CMe_2	H	—	—	16	7	7
6	O	CH_3	—	—	14	5	14
7	S	CH_3	—	—	4	2	10
 **2, 8-11**							
2 (AGN 193491)	CMe_2	H	H, H	H	27	1020	3121
8 (AGN 193618)	CMe_2	H	H, F	H	5.7	622	863
9 (AGN 194202)	CMe_2	H	F, F	H	32	2256	>30 000
10 (AGN 194301)	O	CH_3	H, F	Br	2.8	320	7258
11 (AGN 194574)	O	CH_3	F, F	Br	1.5	898	10 618

Figure 7: *Binding affinities of chromene and tetrahydronaphthalene RAR antagonists, showing structural effects on selectivity. Adapted from Noman et al. [40].*

Pyrazole and pyrrole-based RAR antagonists highlight additional strategies for receptor inhibition. Transitioning from agonists (e.g., compound 12) to antagonists (e.g., ER-27191) reveals how steric modifications prevent receptor activation. Compound 15 demonstrates the highest RARα selectivity with an IC_{50} of 0.5 nM, confirming that molecular refinements can significantly impact receptor targeting.



		Binding affinity IC_{50} (nM)			Antagonism IC_{50} (nM)
Compound	**R**	**RARA**	**RARB**	**RARG**	
15	MeO	0.5	247	350	1.6
16	Et	0.5	17	9	17
17 (ER 50891)	Ph	1.2	278	181	6.4

Figure 8: *Structures of pyrazole and pyrrole-based RAR ligands, illustrating steric and electronic effects on receptor selectivity. Adapted from Noman et al. [40].*

Ongoing developments in RAR inhibitor design focus on refining steric constraints, optimizing electronic properties, and improving ligand-receptor compatibility. These strategies lay the groundwork for the rational design of next-generation RARα inhibitors with therapeutic potential.

Chemical Composition and Structure of YCT-529

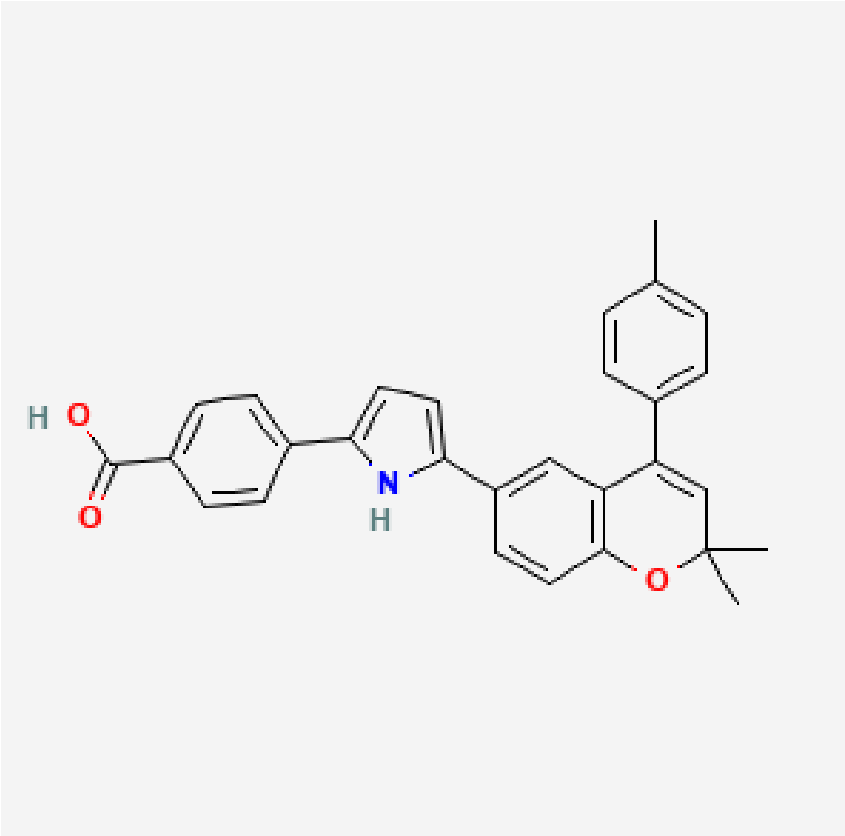


Figure 9: *Chemical structure of YCT-529, featuring a chromene core, a pyrrole ring, and a benzoic acid moiety. The 2,2-dimethyl and 4-methylphenyl substituents contribute to hydrophobicity, while the benzoic acid serves as a hydrogen bond donor, crucial for selective antagonism of RAR-α. Adapted from NCBI [35].*

YCT-529, a small-molecule antagonist of the retinoic acid receptor alpha (RAR-α), is structurally optimized for selective inhibition. Its molecular framework, shown in Figure 9, consists of a chromene core fused to a pyrrole ring and a benzoic acid moiety. The IUPAC name is 4-[5-[2,2-dimethyl-4-(4-methylphenyl)chromen-6-yl]-1H-pyrrol-2-yl]benzoic acid, with a molecular formula of $C_{29}H_{25}NO_3$ and a molecular weight of 435.5 g/mol. YCT-529 is also formulated as a sodium salt to enhance aqueous solubility [50], though its pharmacodynamic properties remain identical to the free acid form.

The chromene core provides the hydrophobic scaffold essential for receptor binding, featuring 2,2-dimethyl and 4-methylphenyl substituents that increase lipophilicity and membrane permeability. These properties contribute to a Log D of 3.5, an XLogP3-AA value of 6, and a solubility of 79 µM at physiological pH, supporting efficient oral bioavailability [50]. The planar aromatic structure enables π-π stacking interactions with RAR-α's ligand-binding domain (LBD), stabilizing the antagonist-receptor complex.

The benzoic acid moiety plays a pivotal role in selectivity. The carboxyl (-COOH) group functions as a hydrogen bond donor and acceptor, forming electrostatic interac-

tions with polar residues in RAR-α's LBD. This enhances specificity, locking the receptor in an inactive state by preventing coactivator recruitment and downstream gene activation. The electron-withdrawing nature of the carboxylic acid also stabilizes the bound conformation, ensuring effective inhibition of RA signaling [50].

The pyrrole ring further strengthens receptor affinity through hydrogen bonding interactions, while its rigid fused-ring system enhances selectivity by restricting conformational flexibility, minimizing off-target effects on RARβ and RARγ. This precise geometric fit ensures strong binding to RAR-α, reinforcing YCT-529's function as a highly selective antagonist.

Biological Function and Chemical Modifications for Selectivity

YCT-529 functions as a selective RAR-α antagonist, interfering with retinoic acid (RA) signaling, which is essential for spermatogenesis [40]. The molecule's high specificity is driven by its rigid fused-ring system, which enforces a precise fit within the receptor's ligand-binding domain (LBD), minimizing off-target interactions with RARβ and RARγ. The chromene-pyrrole scaffold, along with steric hindrance from 2,2-dimethyl and 4-methylphenyl substituents, prevents endogenous ligands from binding. The pyrrole nitrogen acts as a hydrogen bond donor or acceptor, stabilizing interactions with key residues in the LBD, enhancing affinity and potency. Additionally, metabolic resistance is improved by steric shielding, limiting enzymatic degradation and extending the compound's half-life [50]. Preclinical studies confirm YCT-529's stability, with no rapid hepatic metabolism or major biotransformation, ensuring sustained activity.

Empirical receptor-binding assays demonstrate that YCT-529 exhibits higher binding affinity for RAR-α over RARβ and RARγ, similar to structurally related compounds (Figure 7). Compound 11 (AGN 194574) shows strong selectivity with an inhibition constant(K_i) of 1.5 nM for RARα compared to 898 nM and 10,618 nM for RARβ and RARγ, respectively. The lower the value of the constant, the stronger the binding affinity of the inhibitor [40]. So by analogy, YCT-529 is hypothesized to display a comparably low K_i for RAR-α, reinforcing its specificity. Cellular assays measuring downstream gene expression further confirm selective inhibition of RA signaling, as indicated by suppression of *Stra8* expression in spermatogonial stem cells. Off-target toxicity screening, including hERG activity, Ames mutagenicity, and HepG2 cytotoxicity assays, revealed no significant adverse effects, supporting its favorable safety profile [50].

Further in vivo studies assess YCT-529's pharmacodynamic selectivity by evaluating its effects on spermatogenesis without disrupting other RA-regulated physiological processes such as skin homeostasis. Murine models administered YCT-529 show a targeted reduction in sperm production with no observable off-target effects in non-reproductive tissues [50]. Structural analysis using X-ray crystallography and cryo-EM further eluci-

dates YCT-529's binding conformation, confirming that steric hindrance and hydrogen bonding interactions maintain selective antagonism [40]. These findings reinforce YCT-529's potential as a safe, reversible male contraceptive with strong receptor specificity.

Experimental Validation of Spermatogenesis Inhibition

To validate YCT-529's efficacy in inhibiting spermatogenesis, Dr. Georg and colleagues conducted preclinical trials using murine models [50]. Oral administration was selected to mimic real-world contraceptive use, ensuring systemic distribution and high compliance [40]. Mice received varying doses of YCT-529, followed by histological examination of the testes to assess spermatogenesis. The pharmacokinetic (PK) profile revealed a half-life of 11 hours and peak plasma levels of 2.1 µM, indicating sustained systemic exposure. Notably, testicular tissue accumulation was confirmed via distribution studies, ensuring targeted action within reproductive organs [50]. These findings reinforced YCT-529's potential as a non-hormonal contraceptive with effective bioavailability.

Experimental endpoints included sperm count reduction, motility assessment, and germ cell morphology. Results demonstrated a 99% reduction in sperm production, consistent with prior RAR antagonist studies like BMS-189453, confirming that selective antagonism of RAR-α effectively disrupts spermatogenesis [40]. Importantly, fertility was fully restored within 4 weeks post-treatment cessation, supporting YCT-529's reversibility [40]. The compound's distinct structure ensured high selectivity for RAR-α, minimizing off-target effects and preserving testosterone levels. These findings validate YCT-529 as a promising candidate for male contraception, demonstrating both efficacy and reversibility critical for further clinical development.

Preclinical Data Analysis

The preclinical data provided critical insights into YCT-529's targeted action on spermatogenesis. Histopathological analysis of treated mice revealed disrupted spermatid alignment and release, resembling phenotypes observed in RARα knockout models [40]. A key aspect of the study was evaluating reversibility, an essential feature for contraceptive viability. Mice administered 10 mg/kg for 4 weeks or 20 mg/kg for 2 weeks exhibited a 99% reduction in sperm count, with full fertility recovery observed within 4 weeks post-treatment [50]. These findings confirmed that YCT-529 induces a reversible contraceptive effect without permanent impairment of spermatogenesis.

Further analysis showed that YCT-529 did not alter testosterone levels, ensuring preservation of androgen-driven functions such as libido and secondary sexual characteristics. Unlike BMS-189453, which antagonizes all RAR isoforms and can cause systemic toxicity, YCT-529's RAR-α selectivity minimizes off-target effects, contributing to its superior safety profile [50]. The absence of adverse effects in non-reproductive tissues sug-

gests a favorable pharmacological profile. This specificity, driven by YCT-529's unique structure and high receptor affinity, positions it as an improved alternative to earlier RAR antagonists, reinforcing its potential as a safe, reversible male contraceptive.

Retinol, Retinoic Acid (RA) Signaling Pathway and Gene Regulation

To fully understand RAR antagonists like YCT-529, it is essential to examine retinoic acid (RA) signaling. RA regulates spermatogenesis by activating nuclear receptors that control gene expression. YCT-529 selectively inhibits RARα, disrupting these pathways and providing a targeted, non-hormonal contraceptive approach.

Retinol & Retinoic Acid (RA)

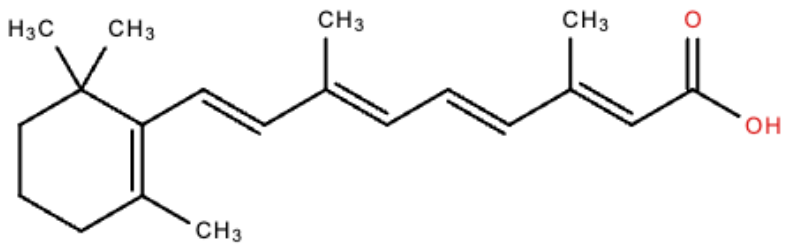


Figure 10: *Chemical structure of retinoic acid, highlighting its terminal carboxyl (-COOH) and polyene chain. The carboxyl enhances polarity and solubility, while conjugated π-electrons stabilize molecular conformation, enabling precise binding to nuclear retinoic acid receptors (RARs). Adapted from [16].*

Vilhais-Neto and Pourquié [60] describe retinoic acid (RA) as a small, lipophilic molecule (300 Da) synthesized from vitamin A (retinol). Figure 10 depicts its structure, consisting of a conjugated polyene chain and a terminal carboxyl (-COOH) group. The π-electrons in the polyene system provide molecular stability, ensuring precise receptor binding, while the carboxyl contributes to RA's polarity ($pKa \approx 4.5$), facilitating solubility and stabilizing the conjugate base. RA differs structurally from retinol (-OH) and retinal (-CHO), which interconvert through sequential redox reactions catalyzed by retinol dehydrogenases (RODHs) and retinal dehydrogenases (RALDH1/2) [60, 21]. In vertebrates, retinol circulates bound to retinol-binding protein 4 (RBP4), forming an RBP4-ROL complex that enters cells via the RBP receptor STRA6, where it undergoes enzymatic conversion to RA [65]. RA is then transported intracellularly by cellular retinoic acid-binding proteins (CRABPs) to regulate gene transcription [39].

Once synthesized, RA binds nuclear receptors, primarily RARs (α, β, γ), to modulate gene transcription. Niederreither et al. [39] identify all-trans-RA as the principal endogenous ligand for RARs, whereas RXRs preferentially bind 9-cis-RA. However, 9-cis-RA's physiological role remains uncertain, as it cannot rescue RA-deficient phenotypes. The RAR-RXR heterodimer, further discussed below, predominantly relies on all-trans-RA to regulate transcription. RA is essential for embryogenesis, where it directs HOX gene expression to establish the anteroposterior body axis and organ patterning [60]. Additionally, RA supports immune function, epithelial integrity, and male fertility by governing spermatogenesis [40]. To prevent excess signaling, cytochrome P450 26 (CYP26) enzymes catalyze RA catabolism, converting it into polar metabolites for elimination, ensuring strict regulation of bioavailability [39].

Retinoic Acid Signaling

In testicular tissue, retinoic acid (RA) synthesis occurs in situ from retinol (ROL), sourced from retinyl ester stores within Sertoli cells or absorbed from circulation bound to retinol binding protein (RBP4). Retinol is biologically inactive and requires conversion into RA via sequential oxidation. First, ROL is transformed into retinaldehyde through retinaldehyde dehydrogenases (RDHs) and cytosolic alcohol dehydrogenases (ADHs). RDH10 is the predominant RDH in the testis, playing a crucial role in RA synthesis, as demonstrated by Sandell et al., where RDH10 knockout in mice resulted in defects in limb, craniofacial, and organ development [47]. In the testis, ADH1 and ADH3 are expressed in Sertoli cells, while ADH4 is found in late spermatocytes [65]. Retinaldehyde then undergoes irreversible oxidation to RA, primarily catalyzed by retinaldehyde dehydrogenases RALDH1 and RALDH2, encoded by ALDH1A1 and ALDH1A2. These enzymes are essential for RA production in the testicular environment [21]. Specifically, ALDH1A1 and ALDH1A2 in Sertoli cells initiate spermatogenesis, as shown by their deletion, which blocked the transition from undifferentiated A spermatogonia to A1 spermatogonia. This spermatogenic blockade was reversible with exogenous RA administration, emphasizing the necessity of RA synthesis in Sertoli cells [65].

Further genetic evidence implicates the autophagy-related gene Atg5 in RA regulation. Deletion of Atg5 in Sertoli cells leads to decreased ALDH1A1 levels and reduced RA synthesis, severely impairing spermatogenesis. This suggests that autophagic processes modulate RA biosynthesis, possibly by maintaining ALDH1A1 expression and activity [65]. The regulation of RA synthesis involves a network of genes, including RDH10, ALDH1A1, ALDH1A2, and Atg5, ensuring precise control necessary for spermatogenesis. Disruptions in these pathways can impair RA synthesis, contributing to spermatogenic defects.

Spermatogenesis

Spermatogenesis is a highly regulated process occurring within the seminiferous tubules, divided into spermatogonial proliferation, meiosis, and spermiogenesis [46]. It begins in neonatal mice with the transition of gonocytes into undifferentiated and differentiated spermatogonia [6]. Spermatogonial stem cells (SSCs) either self-renew or differentiate into primary spermatocytes, initiating meiosis. This transition is controlled by the retinoic acid (RA) signaling pathway, which regulates genes such as *Stra8* and *Rec8* [46]. RA activates nuclear RA receptors (RARs), which bind to RA response elements (RAREs) in the promoters of target genes, inducing transcriptional activation essential for meiotic progression. During meiosis, chromosomal pairing and recombination ensure proper segregation, tightly regulated at both transcriptional and post-transcriptional levels. The final phase, spermiogenesis, involves morphological transformations driven by non-coding RNAs such as microRNAs (miRNAs), piRNAs, and long non-coding RNAs (lncRNAs), which modulate mRNA stability and translation [62].

Sertoli cells form the blood-testis barrier and provide biochemical support to germ cells, regulating spermatogenesis through paracrine signaling and hormonal control. They express key genes such as *Etv5* and *GDNF*, maintaining the SSC niche and directing the balance between self-renewal and differentiation. Follicle-stimulating hormone (FSH) modulates Sertoli cell activity, further influencing germ cell development. RA signaling continues to play a central role, as RAR-RXR heterodimers regulate gene transcription by binding to RAREs in promoters of differentiation-associated genes. This integration of transcriptional regulation, epigenetic modifications, and endocrine signaling ensures that spermatogenesis progresses in a highly coordinated manner to sustain male fertility.

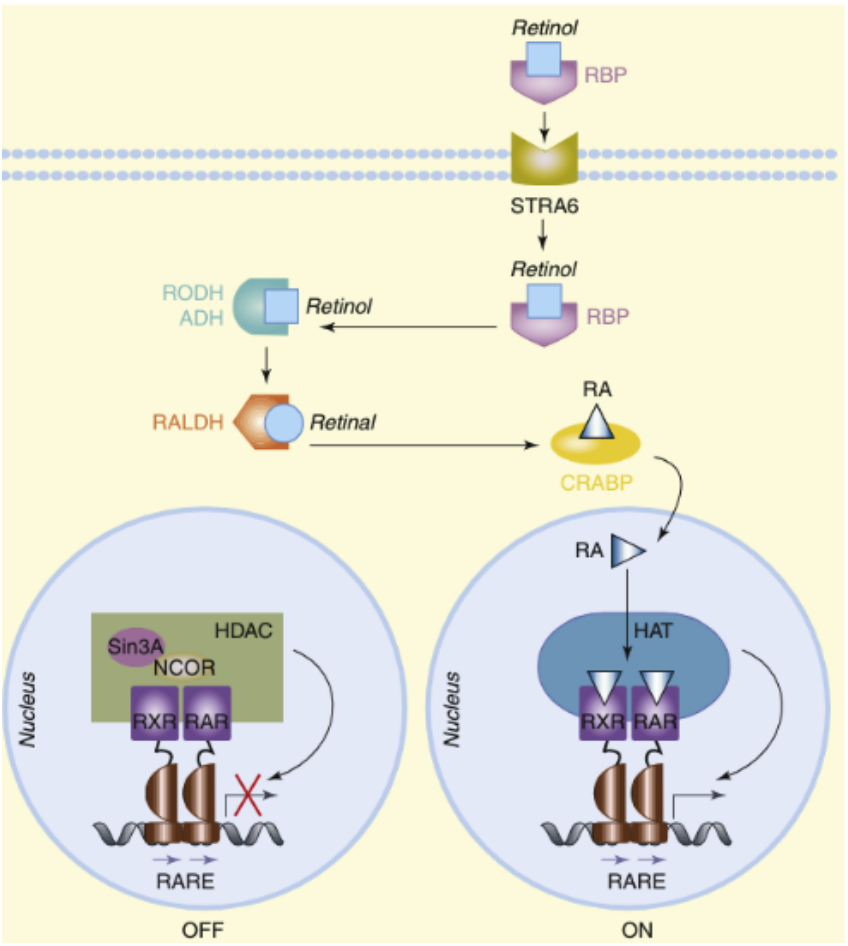


Figure 11: *The cellular function of retinoic acid illustrating its role in gene regulation. Adapted from [60].*

Retinoic Acid's Role in Spermatogenesis

Retinoic acid (RA) plays a crucial role in regulating spermatogenesis, particularly in murine models where it has been extensively studied. RA synthesis in the testes involves a two-step oxidative process mediated by the enzymes RDH10 and ALDH1A, including ALDH1A1, ALDH1A2, and ALDH1A3 [48]. RA acts as a key regulatory molecule throughout the different stages of spermatogenesis, beginning with the differentiation of spermatogonia, thereby exemplifying its role in gene regulation during this process.

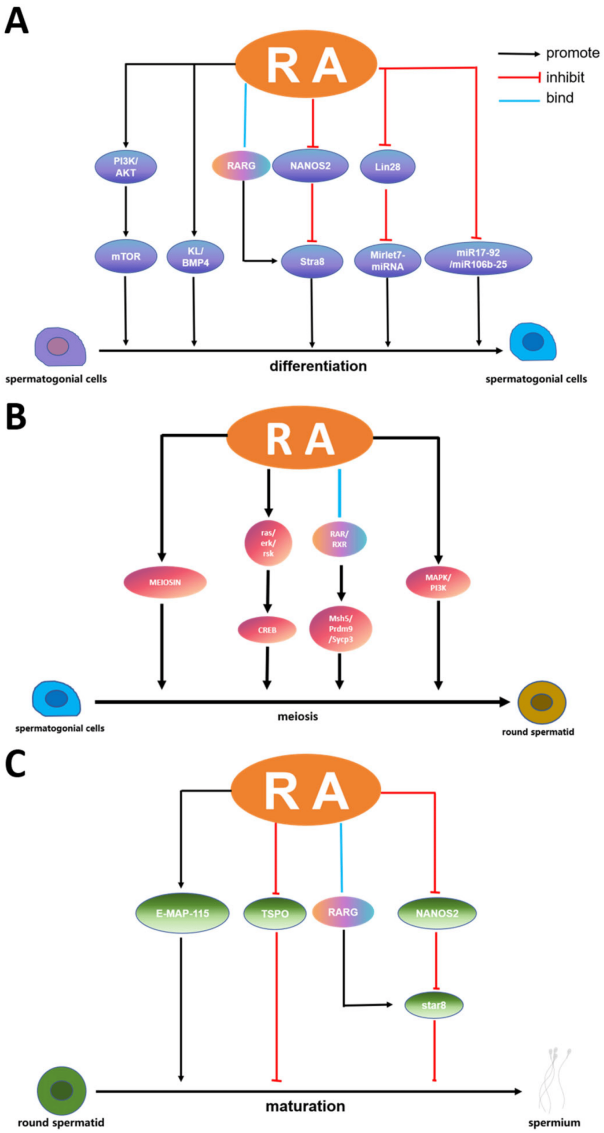


Figure 12: *The role of retinoic acid (RA) in regulating spermatogonia differentiation, meiosis, and maturation. (A) RA promotes spermatogonia differentiation by activating the PI3K/AKT/mTOR pathway, inducing KL and BMP4 expression, and suppressing NANOS2 while upregulating Kit and Stra8. (B) During meiosis, RA activates Kit and induces the expression of genes like Msh5, Prdm9, and Sycp3. (C) In the maturation stage, RA regulates proteins like E-MAP-115 and reduces TSPO and NANOS2 expression, ensuring full sperm development. Adapted from [65].*

Retinoic Acid's Role in Differentiation of Spermatogonia

Retinoic acid (RA) is a key developmental signal that drives the transition of undifferentiated spermatogonial stem cells (SSCs) into differentiated spermatogonia. This process is governed by a complex interplay of spatially and temporally regulated gene expression events. RA, upon binding to its nuclear receptors—RA receptor (RAR) and retinoid X receptor (RXR)—forms a heterodimeric complex that binds to RA response elements (RAREs) in the promoters of target genes, modulating their transcription. The differential expression of genes at specific stages is critical for orchestrating the development of

germ cells. A primary pathway activated by RA is the PI3K/Akt/mTOR kinase signaling cascade, which promotes the proliferation and differentiation of spermatogonial cells [6]. In the absence of RA, as observed in models with long-term vitamin A deficiency or treatment with RA inhibitors like WIN18446, spermatogonia are arrested in an undifferentiated state, failing to progress beyond the Aal (Asynchronous aligned spermatogonia) stage, thus leading to infertility [65]. Supplementation with RA restores this developmental pathway, enabling spermatogonia to progress to the A1 stage and further promoting the downstream stages of spermatogenesis [44]. This demonstrates RA's role in the spatial and temporal regulation of key genes such as *Kit* (encoding the Kit receptor) and *Stra8* (stimulated by retinoic acid gene 8), which are essential for spermatogonial differentiation. RA induces the expression of *Kit* and bone morphogenetic protein 4 (BMP4) in Sertoli cells, while simultaneously inhibiting the expression of glial cell line-derived neurotrophic factor (GDNF), which maintains SSCs in an undifferentiated state [65]. This regulation highlights how RA fine-tunes the balance between SSC self-renewal and differentiation, ensuring the proper progression of spermatogenesis.

In addition to transcriptional regulation, RA exerts control over post-transcriptional gene regulation, influencing the expression of microRNAs (miRNAs) and RNA-binding proteins. For instance, RA suppresses the expression of *Lin28*, which in turn leads to the upregulation of *Mirlet7*—a miRNA crucial for spermatogonial differentiation [66]. Furthermore, RA downregulates the *miR-17-92* (*Mirc1*) and *miR-106b-25* (*Mirc3*) clusters while upregulating genes such as *Bim* (*Bcl2l11*), *Kit*, *Socs3*, and *Stat3*, which are essential for cell differentiation and proliferation (Figure 10A). This combinatorial control of gene expression ensures the appropriate temporal progression and spatial organization of gene expression patterns necessary for proper spermatogenesis. Additionally, RA inhibits the expression of *NANOS2*, an RNA-binding protein that silences *Stra8* and maintains SSCs in an undifferentiated state. By downregulating *NANOS2*, RA facilitates the entry of spermatogonia into meiosis, exemplifying how differential gene expression is crucial for the transition between distinct developmental stages [54].

The dynamic interplay between RA and miRNAs exemplifies the regulatory networks underlying gene expression in spermatogenesis. The *miR-17-92* (*Mirc1*) cluster, which is highly expressed in early germ cells, gradually decreases as cells mature, allowing for the precise timing of developmental transitions. Tong et al. [56] demonstrated that knockout of *Mir-17-92* (*Mirc1*) in male germ cells leads to small testes, reduced sperm count, and defects in spermatogenesis. This knockout resulted in a compensatory increase in *miR-106b-25* (*Mirc3*) cluster miRNAs [56, 65]. This interplay between RA, miRNAs, and other regulatory factors underscores the multifaceted mechanisms by which RA regulates spermatogonial differentiation. RA-mediated regulation of gene expression, involving both transcriptional activation and post-transcriptional modulation, orchestrates the precise control of spermatogenesis and germ cell development [65]. By directing the tem-

poral sequence and spatial distribution of gene expression events, RA ensures the orderly progression from SSCs to mature spermatozoa, illustrating its role in the developmental processes of multicellular organisms.

Retinoic Acid in the Initiation of Meiosis in Spermatogenic Cells

As spermatogonia enter meiotic prophase, retinoic acid (RA) orchestrates a complex network of molecular pathways essential for this transition, serving as a master regulator of gene expression. A key gene activated by RA is *Stra8*, which is crucial for initiating meiosis. At low concentrations (10^{-8} mol/L), RA induces the expression of *Stra8*, and its absence leads to an accumulation of undifferentiated spermatogonia, as observed in *Stra8*-null mice [65]. The activation of *Stra8* exemplifies the role of RA in gene regulation, where RA binds to its receptors, RAR and RXR, forming a heterodimeric complex that interacts with RA response elements (RAREs) in the promoter region of the *Stra8* gene to modulate its transcription. This activation is tightly regulated by transcriptional repressors like *DMRT1*, which inhibits RA signaling and the expression of *Stra8*, acting as a checkpoint to prevent premature entry into meiosis. In parallel, *DMRT1* upregulates *Sohlh1*, a gene that promotes spermatogonial maturation while delaying meiosis onset [34, 65].

RA also influences the expression of PLZF, a transcriptional repressor that maintains spermatogonial stem cells (SSCs) in an undifferentiated state. By downregulating PLZF, RA removes this inhibitory signal, allowing SSCs to differentiate and progress toward meiosis [65]. Furthermore, RA triggers the phosphorylation of the Kit receptor through the activation of MAPK and PI3K pathways (Figure 10B). The phosphorylated Kit receptor then promotes the mitosis-to-meiosis transition by facilitating DNA synthesis and cell cycle progression [65]. The interaction between RA and follicle-stimulating hormone (FSH) adds another layer of regulatory control. FSH regulates the expression of ALDH1A2, the enzyme that converts retinal to RA as discussed above, thereby indirectly modulating RA levels within the testes [51, 65]. FSH further enhances the sensitivity of spermatogonia to RA by upregulating the expression of RARα in both spermatogonia and Sertoli cells, increasing cellular responsiveness to RA and promoting the initiation of meiosis. Additionally, FSH regulates the expression of GFRα1 in Sertoli cells, thereby influencing the self-renewal and differentiation balance of spermatogonia [65]. This intricate regulatory network, where RA modulates gene expression at multiple points, ensures the precise timing and progression of spermatogenesis.

Retinoic Acid's Role in the Late Meiotic Phase of Spermatogenesis

In the late meiotic phase, RA continues to be a critical regulator, ensuring proper chromosomal segregation and maturation of spermatogenic cells. RA regulates the expression of

genes crucial for chromosomal pairing, recombination, and segregation, including *Msh5*, *Prdm9*, and *Sycp3*, through the RAR and RXR receptors [62, 65]. *Msh5* is involved in homologous chromosome pairing and recombination, while *Prdm9* facilitates chromosomal segment exchange during recombination. *Sycp3* plays a key role in chromosome pairing and synapsis, illustrating how RA-mediated regulation of these genes ensures the accurate transition of spermatocytes from the diploid to haploid state. Additionally, RA activates protein kinase C, which signals through the ras/ERK/RSK pathway independently of RARs to activate CREB (cAMP response element-binding protein), a critical transcription factor for genes involved in the late meiotic phase (Figure 10C).

RA also interacts with cAMP signaling during this phase, particularly through the activation of CREM (cAMP response element modulator), which regulates transcriptional activation in late meiotic germ cells. Loss of CREM function results in increased apoptosis during the round spermatocyte stage, indicating its role in cell survival and meiotic progression [65]. RA directly influences the expression of MEIOSIN, a crucial protein encoded by the *Gm4969* gene, which collaborates with *Stra8* to coordinate the timing of meiosis [24, 65]. MEIOSIN expression is stage-specific, occurring particularly during stages VIII-IX of the spermatogenic cycle, when RA levels are elevated [24]. Inhibition of RA signaling, for example by the use of WIN18446, suppresses MEIOSIN expression, halting spermatogenesis in the late meiotic phase. Reactivation of MEIOSIN through exogenous RA treatment further underscores the indispensability of RA in the regulation of meiotic progression and the maturation of spermatogenic cells [65]. This demonstrates RA's multifaceted role in gene regulation, where it modulates various signaling pathways and transcriptional networks to ensure the completion of meiosis and the successful production of spermatozoa.

Retinoic Acid's Role in the Final Stages of Spermatogenesis

During the final stages of spermatogenesis, retinoic acid (RA) plays a pivotal role in the spatial and temporal regulation of gene expression necessary for the formation and release of spermatozoa. Central to this process is the identification of key genes regulated by RA, which orchestrates a developmental cascade by modulating their expression to facilitate sperm release from the seminiferous tubules. A critical example is the identification of the gene encoding E-MAP-115, a protein essential for sperm release [26]. Using gene identification approaches, researchers have elucidated that in vitamin A deficiency (VAD) conditions, where RA synthesis is impaired, spermatozoa are abnormally retained within the spermatogenic epithelium. This observation not only underscores the necessity of RA for proper temporal release into the tubule lumen but also highlights the importance of identifying specific RA-regulated genes, such as E-MAP-115, that are crucial for this spatially regulated release process [19, 65].

Further gene identification efforts have focused on genes like *Stra8* and *NANOS2*. The RA-induced expression of *Stra8* is a key marker for spermatogonia differentiation, highlighting how gene identification contributes to understanding the early stages of spermatogenesis. Conversely, as mentioned above, RA suppresses the expression of *NANOS2*, enabling spermatocytes to progress toward the formation of mature spermatids [54]. The spatial and temporal regulation of these genes, identified through molecular and genetic approaches, illustrates how differential gene expression facilitates the orderly development of germ cells and underscores the integral role of RA in this regulation.

RA's interaction with its receptors, particularly RARα, is crucial for the spatial organization and temporal progression of sperm maturation. Gene identification methods have been instrumental in revealing how the absence of RARα results in defects such as impaired spermatid translocation, abnormal morphology, and failure of spermatozoa release [59]. These findings underscore the role of RA in ensuring the coordinated expression of identified genes at precise times and locations within the seminiferous epithelium. The use of knockout models to "lose" specific RA-regulated genes has provided further evidence of the spatial and temporal regulation mechanisms at play. In RARα knockout models, for instance, overexpression of RA can restore spermatogenesis, indicating that RA acts through the precise temporal regulation of key genes identified as crucial for differentiation [65]. This includes the transition of undifferentiated (A_{undiff}) spermatogonia into A1 spermatogonia and the initiation of meiosis. The disruption of RA synthesis—through the targeted ablation of genes such as *RDH10* and *ALDH1A*—results in the spatial accumulation of undifferentiated spermatogonia, reflecting the developmental consequences of altered spatial gene expression patterns [65].

A hallmark of RA's role in spermatogenesis is its pulsatile nature, which illustrates the temporal regulation necessary for the continuous cycle of sperm production. In adult mice, RA is synthesized in pulses every 8.6 days, driving the differentiation of spermatogonia through sequential stages from A1 to B. This pulsatile mechanism ensures that different germ cells are exposed to RA at specific developmental stages, promoting synchronized spermatozoa release and maintaining fertility [65]. This discovery, facilitated by identifying the temporal synthesis and degradation of RA, demonstrates the finely tuned temporal regulation that ensures spermatogenesis proceeds in an orderly and synchronized manner, allowing germ cells to progress through various stages in a controlled fashion.

In humans, RA's role in spermatogenesis is similarly critical, although the regulatory mechanisms exhibit species-specific differences. Gene identification in human spermatogonial development has revealed unique cell types, such as dark type A spermatogonia (Ad), pale type A spermatogonia (Ap), and B spermatogonia, reflecting a more complex regulatory landscape [48]. Through the identification and study of genes involved in these processes, RA has been shown to regulate not only spermatogonia differentia-

tion but also the maintenance of the blood-testis barrier (BTB). RA's interaction with retinoic acid receptors (RARs) and retinoid X receptors (RXRs) in Sertoli cells facilitates BTB formation, highlighting how RA-mediated regulation of identified genes contributes to maintaining a supportive microenvironment within the testes. Disruptions in RA-RAR/RXR signaling, observed in models with ablated RXRβ in Sertoli cells, result in compromised BTB integrity, impacting the temporal and spatial aspects of spermatogenesis [48]. These disruptions lead to abnormalities in sperm release and maturation, further illustrating how spatially and temporally regulated differential gene expression, informed by gene identification efforts, is vital for normal development.

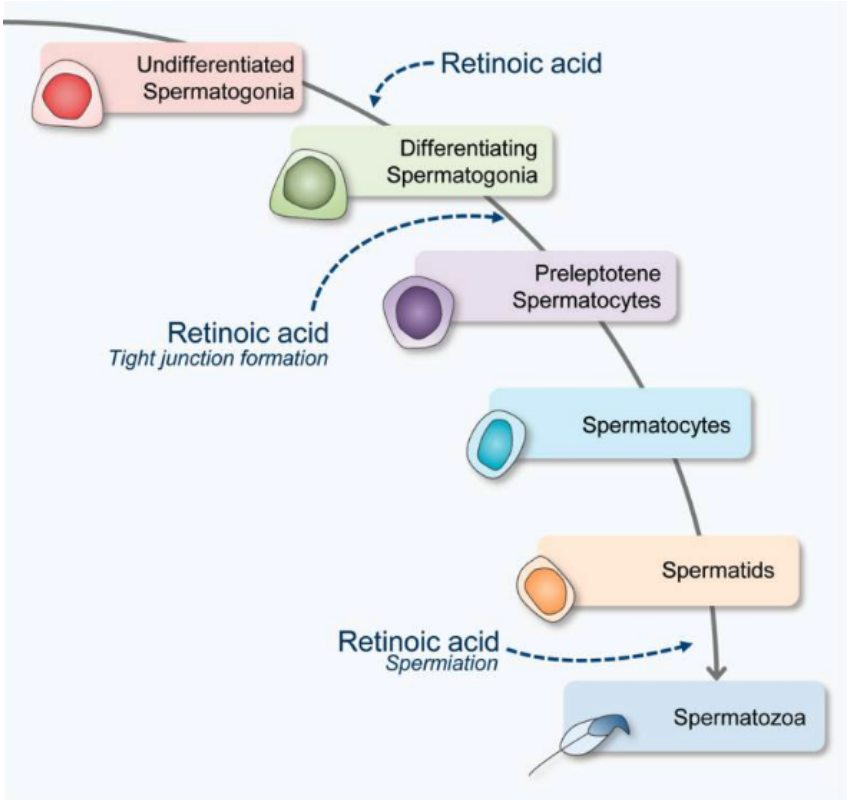


Figure 13: *The role of retinoic acid (RA) throughout spermatogenesis. RA is crucial at multiple stages, including the differentiation of undifferentiated spermatogonia into differentiating spermatogonia, the formation of tight junctions during preleptotene spermatocyte development, and the process of spermiation, where mature spermatids are released as spermatozoa. This illustrates the essential regulatory function of RA in both the progression and maturation of germ cells [48].*

RA's involvement in spermatogenesis exemplifies the "find it, lose it, move it" strategy in developmental genetics. Identifying RA and its receptors in the testis ("find it") highlights the spatial expression pattern crucial for germ cell development, where gene identification efforts have pinpointed critical targets regulated by RA. Disruption of RA synthesis enzymes like RDH10 or receptors such as RARα ("lose it") reveals how alterations in temporal gene expression, identified as crucial through targeted genetic approaches, can halt spermatogenesis, emphasizing RA's necessity. "Move it" experiments,

where exogenous RA restores spermatogenesis, demonstrate RA's sufficiency in regulating the spatial and temporal aspects of germ cell development [65]. These findings underscore RA's central role in differential gene expression, maintaining the complex developmental processes in the final stages of spermatogenesis, and highlight the importance of gene identification in uncovering the molecular underpinnings of RA-mediated regulation.

Receptor Inhibition

Receptor inhibition occurs when a molecule binds to a receptor, preventing its activation and altering cellular function. This interaction is governed by the structural complementarity between the inhibitor and the receptor's binding site, ensuring high affinity and specificity. Inhibitory receptors often exhibit stronger ligand binding compared to coactivating receptors due to their conformational arrangement, which stabilizes the inhibitory molecule [28]. Receptor antagonists structurally mimic natural ligands, occupying the active site without triggering receptor activation, thereby blocking function. Depending on their binding characteristics, antagonists can be reversible, forming transient interactions that allow the receptor to regain activity, or irreversible, forming covalent bonds that permanently inactivate the receptor [29]. Additionally, pseudo-irreversible antagonists dissociate slowly due to their structural properties, prolonging inhibition [29]. These distinctions in binding kinetics underscore how the molecular architecture of inhibitors influences receptor function, demonstrating the principle that biological activity is inherently linked to structure.

RARα Inhibition and the Creation of YCT-529

Retinoic Acid Receptor Inhibition and Mechanism

Retinoic acid receptors (RARs), particularly RARα, regulate gene transcription in response to retinoic acid (RA). Structurally, RARα consists of a ligand-binding domain (LBD) that accommodates RA and a DNA-binding domain (DBD) that interacts with retinoic acid response elements (RAREs) on DNA. In the absence of RA, the RAR-RXR heterodimer associates with corepressors such as NCOR1 and NCOR2, which recruit histone deacetylases (HDACs) and histone methyltransferases, leading to chromatin compaction and transcriptional repression [60, 39]. This regulation is mediated by histone modifications, including H3K9, H3K27, and H4K20 methylation, which restrict transcription factor access to DNA. Upon RA binding, RARα undergoes a structural rearrangement that repositions helix 12 in the LBD, displacing corepressors and allowing coactivators such as NCOA1 and NCOA2 to bind. These coactivators exhibit histone acetyltransferase (HAT) activity, promoting histone acetylation at H3K4, H3K9, and H4K14, facilitating chromatin remodeling and transcriptional activation [50].

RAR antagonists like YCT-529 interfere with this mechanism by stabilizing RARα in an inactive conformation, preventing the displacement of corepressors and coactivator recruitment. YCT-529 binds to the LBD, locking helix 12 in an inactive state, thereby blocking the conformational shift required for transcriptional activation. This structural interference sustains the association of NCOR1/NCOR2 and HDACs, ensuring continued chromatin repression even in the presence of RA [50]. Studies confirm that YCT-529 maintains the transcriptionally repressed state, effectively inhibiting RA-mediated gene expression critical for spermatogenesis [50]. Tissue distribution studies further show that YCT-529 exhibits selective accumulation in the testes, reinforcing its targeted contraceptive action without disrupting RA signaling in other tissues [50].

Antagonists can further stabilize the RAR-RXR-corepressor complex, maintaining prolonged repression of RA-responsive genes. This inhibition prevents the activation of spermatogenesis-associated pathways, demonstrating how YCT-529 precisely modulates gene expression at the molecular level. By directly targeting the structural components of RARα, YCT-529 serves as a potent and selective non-hormonal contraceptive agent. These findings highlight the role of ligand-binding dynamics in regulating transcriptional activity and illustrate how rational drug design can exploit receptor structure to achieve selective inhibition [50, 40].

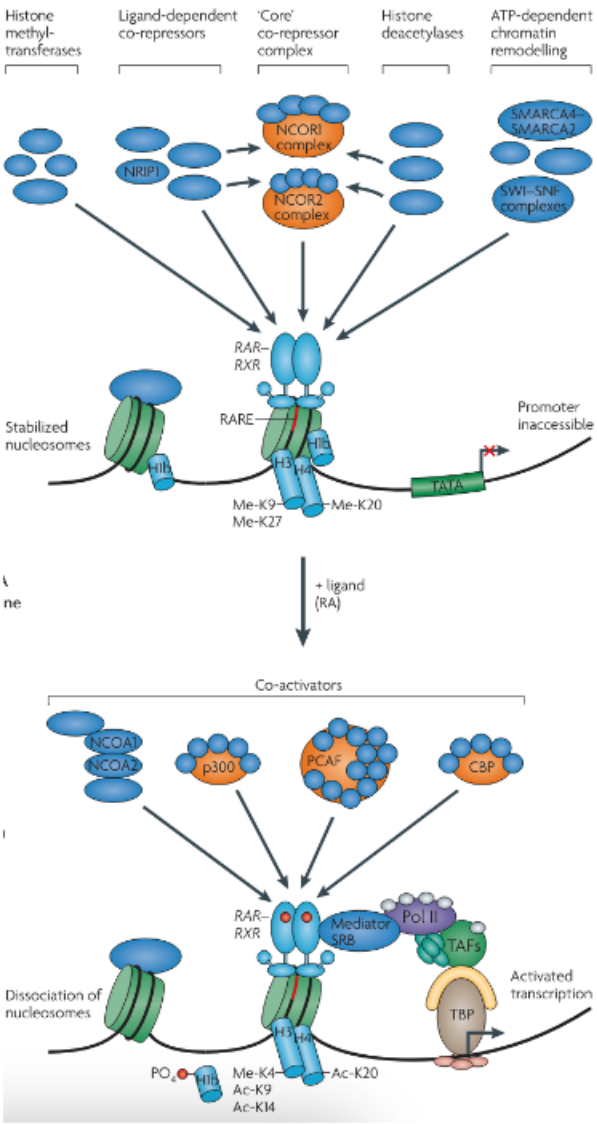


Figure 14: *Mechanism of RAR-mediated gene regulation. In the absence of RA, RAR-RXR heterodimers recruit NCOR1, NCOR2, and HDACs, maintaining chromatin in a repressive state via histone methylation (Me-K9, Me-K27). RA binding induces conformational changes that release corepressors and recruit coactivators such as p300, PCAF, and CBP, which promote histone acetylation (Ac-K4, Ac-K9) and transcriptional activation. YCT-529 prevents these conformational changes by stabilizing helix 12 in an inactive position, blocking coactivator recruitment and sustaining gene repression [50].*

Specificity of RARα, RARβ, and RARγ Inhibition

The mechanisms of antagonism differ among RARα, RARβ, and RARγ due to their distinct ligand-binding properties and tissue distributions. RARα is highly expressed in the testes, where it mediates RA signaling essential for germ cell proliferation and differentiation. Structurally, RARα contains a ligand-binding domain (LBD) optimized for selective RA binding, alongside a DNA-binding domain (DBD) that facilitates interaction with retinoic acid response elements (RAREs) in target gene promoters. Briefly mentioned earlier, notably, RARα's LBD features Ser232, a residue absent in RARβ and

RARγ, which enhances its specificity for ligands like YCT-529 by providing a hydrogen bond donor site critical for selective inhibition [50]. Functional studies reveal that RARα-null mice exhibit sterility due to impaired testicular development, underscoring the necessity of this isoform for male fertility. In contrast, RARβ and RARγ play broader roles in embryogenesis and tissue homeostasis, with RARβ- and RARγ-null males remaining fertile, reinforcing the unique function of RARα in spermatogenesis [65]. RXRβ, a heterodimeric partner of RARs, is also critical for male fertility, as RXRβ-null males exhibit testicular degeneration, further demonstrating the receptor-specific regulation of reproductive processes.

YCT-529's selectivity for RARα stems from its ability to exploit structural differences in the LBD. The presence of Ser232 in RARα provides a polar interaction site, enabling YCT-529 to form strong hydrogen bonds that enhance its binding affinity, while the corresponding positions in RARβ (Ala225) and RARγ (Ala234) lack this feature, reducing affinity for these isoforms [50]. This structural distinction explains why YCT-529 effectively inhibits RA signaling in spermatogenesis while sparing other RA-dependent pathways. RARα inhibition specifically suppresses genes involved in germ cell differentiation and blood-testis barrier maintenance, preventing spermatogonial differentiation and disrupting testicular function. While the core mechanism of inhibition—blocking the LBD and preventing coactivator recruitment—remains consistent across all RAR isoforms, the structural differences in binding affinity dictate their tissue-specific roles. This selectivity underscores the rational design of YCT-529 as a targeted non-hormonal contraceptive, effectively disrupting RA signaling in the testes without affecting other physiological processes [40].

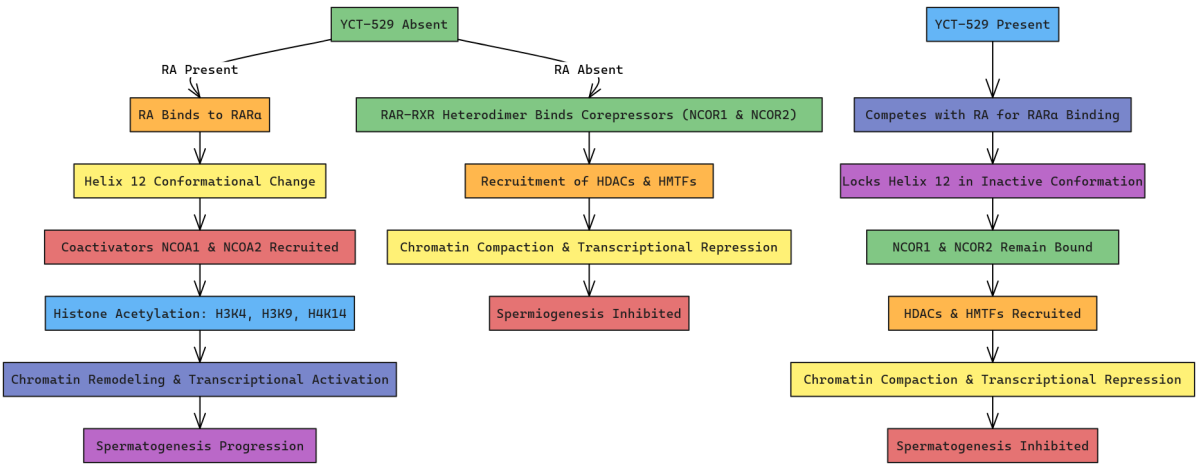


Figure 15: *YCT-529 inhibits RA-induced transcriptional activation by stabilizing RARα in an inactive conformation.*

Impact on Gene Expression and Pathways

Antagonism of retinoic acid receptors significantly affects both upstream and downstream pathways, impacting gene expression and cellular phenotypes. By blocking RAR activity, the transcription of downstream target genes is suppressed, disrupting processes such as cell differentiation and tissue morphogenesis. In spermatogenesis, RARα antagonism prevents the transcription of genes critical for the transition from undifferentiated spermatogonia to mature spermatozoa. This suppression halts the progression of germ cells through meiosis and spermiogenesis, leading to infertility. The absence of RAR activity may induce a feedback increase in RA synthesis as the body attempts to compensate for the lack of transcriptional response, although this compensatory mechanism is not fully understood.

Among the three isoforms, RARα inhibition is necessary and sufficient for the suppression of spermatogenesis, demonstrating its crucial role in the reproductive system. Targeting RARα alone is sufficient to block RA-mediated signaling required for germ cell differentiation, directly affecting the expression of genes that regulate spermatogonial differentiation and the integrity of the blood-testis barrier. Studies have shown that RARα antagonism impacts key molecular pathways, influencing cellular processes like apoptosis, cell cycle regulation, and chromatin remodeling. This emphasizes the link between molecular interactions at the receptor level and the resulting phenotypic outcomes in spermatogenesis. Therefore, the antagonism of RARα plays a pivotal role in male fertility regulation, making it a key target for contraceptive development.

Noman et al. [40] provides a comprehensive overview of the critical functions of RARα, in spermatogenesis. The targeted mutagenesis of the *Rara* gene in *Rara*$^{-/-}$ mice resulted in disruptions in spermatogenesis analogous to the effects observed in Vitamin A deficiency (VAD), leading to complete male infertility while females remained fertile [40, 11]. These *Rara*$^{-/-}$ mice exhibited severe abnormalities in testicular tubules by 4–5 months of age, displaying few germ cells, and the epididymides contained only a minimal number of abnormal spermatids [32]. A chronological study of spermatogenic abnormalities revealed that *Rara*$^{-/-}$ mice had defective spermiation, with late spermatids failing to be released at the end of stage VIII and still present at the luminal edge at stage IX (Figure 5) [10, 63]. Additionally, spermatids underwent apoptosis and were engulfed by Sertoli cells, indicating a failure in the final stages of spermatogenesis [9]. Further investigation into germ-cell-specific *Rara* conditional knockout mice (*Rara* cKO) demonstrated that RARα plays a vital role in modulating the synaptonemal complex during meiosis, supporting previous findings that meiosis is delayed in VAD animals and *Rara*$^{-/-}$ mice [45]. The role of RARα in spermiogenesis was further corroborated by studies showing that targeted expression of *Rara* cDNA in haploid spermatids partially rescued spermiogenic defects in otherwise RARα-deficient mice [10]. Moreover, all-trans

retinoic acid (ATRA) has been implicated in two crucial postmeiotic transitions: the initiation of spermatid elongation and spermiation [17, 40]. For a deeper understanding of the specific functions of retinol and ATRA during spermatogenesis, including their influence on cellular processes within the seminiferous tubules, see [11, 63] for further detailed reviews.

Inferences, Conclusions, and Future Prospects

YCT-529's potent and selective antagonism of RAR-α positions it as a leading non-hormonal male contraceptive. By inducing reversible sperm suppression through targeted RA signaling disruption, it offers a pharmacologically sound approach with minimal systemic effects. Ongoing clinical trials will assess long-term safety, addressing concerns about Vitamin A signaling. If successful, YCT-529 could transform male contraception, bridging scientific innovation with expanded reproductive choices [35].

Next Steps

Clinical Development and Phase 1 Trials

Building on preclinical success, YCT-529 has advanced to Phase 1 clinical trials in London to assess safety, tolerability, pharmacokinetics, and pharmacodynamics (ClinicalTrials.gov ID NCT06094283) [50]. Primate studies demonstrated that daily oral doses of 10–20 mg/kg induced over 99% sperm suppression without systemic toxicity. Conducted as a randomized, double-blind, placebo-controlled study, Phase 1 monitors hematology, liver enzymes, and renal function for systemic safety. A fed-state assessment evaluates food's impact on pharmacokinetics, addressing absorption variability. If results confirm favorable pharmacokinetics and minimal side effects, Phase 2 will refine dosing and assess efficacy. However, adverse events such as hepatotoxicity or endocrine disruption could necessitate reformulation, delaying clinical progression. A successful trial outcome would validate YCT-529's preclinical findings, bolster public confidence, and accelerate regulatory approval. Future Phase 1b/2a trials (NCT06542237) will further evaluate efficacy and safety in a larger cohort.

Prequel to the Research Paper: Predictive Modeling of YCT-529's Pharmacokinetics

This study builds upon computational modeling approaches to predict the pharmacokinetics and ADME properties of YCT-529. Using PBPK simulations in PK-Sim, key parameters such as bioavailability, clearance, and systemic distribution will be evaluated across different populations. Simulations across Black American, East Asian, White American, and European cohorts could reveal dose-dependent systemic retention, with East Asians exhibiting prolonged exposure due to slower CYP-mediated metabolism. Machine learning algorithms integrated with ADMETLab 3.0 wil assess YCT-529's solubility, lipophilicity, and metabolic stability, refining dosage recommendations. These in-silico findings could provide a predictive framework for optimizing YCT-529's therapeutic potential, guiding experimental validation, and supporting its future clinical development.

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